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DOCKET NO: 242650US0CONT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF	:
WOLF-RUEDIGER SCHAEBITZ, ET AL.	: EXAMINER: BORGEEST
SERIAL NO: 10/659,295	:
FILED: SEPTEMBER 11, 2003	: GROUP ART UNIT: 1649
FOR: METHODS OF TREATING NEUROLOGICAL CONDITIONS WITH HEMATOPOIETIC GROWTH FACTORS	:

DECLARATION UNDER 37 C.F.R. §1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

Wolf-Ruediger Schaebitz, Stefan Schwab, and Rainer Kollmar, declare and state that:

1. We are named coinventors of U.S. Serial No. 10/659,295 and are familiar with the prosecution history thereof.

2. Prior to June 7, 2001 we had conceived and reduced to practice the invention using GCSF for the treatment of cerebral ischemia (i.e. stroke), Parkinson's Disease, Alzheimer's Disease, and traumatic brain injury described and claimed in the above-identified U.S. Patent Application.

3. The conception and reduction to practice is supported by the appended German Offenlegungsschrift DE 100 33 219 A1. An English translation of the same is also attached herewith.

4. The undersigned declare further that all statements made herein of their own knowledge are true and that all statements made on information are believed to be true.

Further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Wolf-Ruediger Schaebitz

13.6.07

Date

Stefan Schwab

Date

Rainer Kollmar

Date

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Stefan Schwab

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Wolf-Ruediger Schaebitz

Date

Stefan Schwab

Date

Rainer Kollmar

Date 18 July 07

Use of granulocyte colony stimulating factor as neuroprotective agent, for treating acute ischemia and neurodegenerative diseases

Publication number: DE10033219
Publication date: 2002-01-24
Inventor:
Applicant: UNIV HEIDELBERG (DE)
Classification:
- International: ***A61K38/19;*** **A61K38/19;** (IPC1-7): A61K38/19
- European: A61K38/19B
Application number: DE20001033219 20000707
Priority number(s): DE20001033219 20000707

Abstract of **DE10033219**

The use of G-CSF (granulocyte colony stimulating factor) is claimed in the production of pharmaceutical preparations having neuroprotective activity, for the treatment of acute ischemia and neurodegenerative diseases. An Independent claim is included for a commercial package including a G-CSF-containing pharmaceutical preparation and instructions for the use of G-CSF in the neuroprotective treatment of acute ischemia and neurodegenerative diseases.

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(19) BUNDESREPUBLIK DEUTSCHLAND

DEUTSCHES PATENT- UND MARKENAMT

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(51) Int. Cl.7: A 61 K 38/19

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Erfinder wird später genannt werden

(56) Entgegenhaltungen:

WO 99 17 798 A1
WO 00 04 926 A2
JP 05-2 48 885 A

Die folgenden Angaben sind den vom Anmelder eingereichten Unterlagen entnommen

Prüfungsantrag gem. § 44 PatG ist gestellt

(54) Neuroprotektive Wirkung von Granulocyten-Colony Stimmulierendem Faktor (G-CSF)

(57) Verwendung von G-CSF (Granulozyten Colony Stimulating Factor) zur Herstellung pharmazeutischer Präparate mit neuroprotektiver Wirkung zur Behandlung akuter Ischämien wie z. B. Apoplexie und neurodegenerativer Erkrankungen wie z. B. bei der Parkinson- oder Alzheimer-Krankheit.

DE 100 33 219 A 1

Beschreibung

[0001] Verwendung von G-CSF (Granulozyten Colony Stimulating Factor) zur Herstellung pharmazeutischer Präparate mit neuroprotektiver Wirkung zur Behandlung akuter Ischämien.

[0002] Der Schlaganfall (Apoplexia cerebri) ist die dritthäufigste Todesursache in den westlichen Industrieländern, zählt zu den führenden Ursachen dauernder Invalidität und Pflegebedürftigkeit und damit – ökonomisch betrachtet – zu der teuersten Krankheitsgruppe überhaupt. Zur Zeit erleiden in Deutschland etwa 150.000 Einwohner pro Jahr einen Schlaganfall, davon sterben 15–20 Prozent der Patienten innerhalb der ersten vier Wochen. Nur etwa ein Drittel der überlebenden Patienten erholt sich ohne größere bleibende Behinderung, während ebenfalls ein Drittel durch Lähmungen oder andere neurologische Ausfälle dauerhaft schwer behindert bleibt. Bei 80 Prozent der Patienten liegt dem Schlaganfall eine Durchblutungsstörung mit nachfolgender Ischämie in einem umschriebenen Gefäßterritorium zugrunde. Zu Durchblutungsstörungen im Gehirn kommt es meist entweder makroangiopathisch durch Thromboembolien bzw. hämodynamische Strömungsverlangsamungen oder mikroangiopathisch durch eine blutdruckbedingte Arteriosklerose der kleinen, intrazerebralen Endarterien. Dabei begünstigen eine Reihe von Risikofaktoren das Auftreten eines Schlaganfalles. Dies sind insbesondere die arterielle Hypertonie, zahlreiche Herzerkrankungen, die mit einem erhöhten Embolierisiko verbunden sind – vor allem das Vorhofflimmern –, der Diabetes mellitus, Zigarettenrauchen, Blutgerinnungsstörungen und zu einem geringeren Anteil die Hypercholesterinämie. Durch embolischen oder lokal thrombotischen Verschluss einer der großen hirnversorgenden Arterien entstehen die Territorialinfarkte, d. h. Infarkte, die ein umschriebenes Gebiet innerhalb des Versorgungsbereiches einer bestimmten Hirnarterie betrifft. Am häufigsten ist dabei das Versorgungsgebiet der Arteria cerebri media betroffen, ein Mediaterritorialinfarkt mit einem entsprechenden "Mediasyndrom" entsteht. Dies ist auch die häufigste Manifestation eines Schlaganfalles überhaupt. Bisher ist nur bei ausgewählten Patienten eine thrombolytische Therapie erfolgversprechend. In der letzten Jahren hat sich durch neue pathophysiologische Erkenntnisse und Methoden die Diagnostik und Therapie akuter zerebraler Ischämien erheblich gewandelt. Die Thrombolyse bietet die Möglichkeit zur therapeutischen Intervention innerhalb eines "therapeutischen Fensters" von 3 bis 6 Stunden nach Infarktereignis. Ziel ist die rasche Auflösung des Gefäßverschlusses und damit die Wiederherstellung der zerebralen Durchblutung und Verbesserung der neurologischen Symptomatik. Dies basiert auf der pathophysiologischen Vorstellung, dass die Wiedereröffnung eines verschlossenen zerebralen Gefäßes den Erhalt hypoperfundierten, reversibel geschädigten Hirngewebes (der sogenannten ischämische Penumbra), und damit die Wiederherstellung neuronaler Funktionen unterstützt. Bisher kann diese Behandlung allerdings nur in ausgewiesenen neurologischen Zentren durchgeführt werden. Auch die Zulassung von rt-PA beim Schlaganfall in Deutschland steht noch aus. Die Lysetherapie nach 6 Stunden gilt als besonders nebenwirkungsreich (erhöhte Zahl intrakranieller Blutungen) und sollte daher unterbleiben. Andere Therapieverfahren sind zur Zeit nicht evaluiert. Momentan werden verschiedene andere Substanzen untersucht. Hier sind vor allem sog. Wachstumsfaktoren (bFGF) und Pharmaka, die die Thrombozytenadhäsion blockieren (anti-GP IIb/IIa, Abciximab), zu nennen. In den letzten Jahren wurden zahlreiche neuroprotektive Substanzen in klinischen Studien untersucht. Leider konnte keine dieser bisher getesteten Substanzen, die sämtlich im Tiermodell neuroprotektiv wirkten, in der klinischen Praxis ihren Nutzen beweisen. Vor allem die Glutamat-Antagonisten, freie Radikalenfänger und NMDA Antagonisten blieben ohne klinischen Nutzen, oder zeigten im Gegenteil sogar erhebliche Nebenwirkungen, die den klinischen Einsatz unmöglich machen (Psychosen etc.). Andere Substanzen, die die leukozytäre Wandadhäsion hemmen (anti-ICAM-1) oder der Inhibitor der Glutamat-vermittelten NO Synthetase (Lubeluzol) blieben ohne positiven Effekt.

[0003] Aufgabe der Erfindung ist die Folgen einer akuten Ischämie zu lindern oder zu beseitigen durch Verabreichung von Substanzen mit neuroprotektiver Wirkung. Diese Aufgabe wird durch das Verfahren mit den Merkmalen des Anspruches 1 gelöst.

[0004] Die Verwendung von G-CSF (Granulozyten Colony Stimulating Factor) zur Herstellung pharmazeutischer Präparate mit neuroprotektiver Wirkung zur Behandlung akuter Ischämien stellt einen erfolgreichen Therapieansatz mittels neuroprotektiver Wachstumsfaktoren dar.

[0005] G-CSF, englische Abkürzung für "Granulocyte Colony Stimulating Factor (G-CSF)", reguliert als endogener, hämatopoetischer Wachstumsfaktor die Reifung, Proliferation und Differenzierung von neutrophilen Granulozyten. G-CSF wird natürlicherweise von verschiedenen Monozyten, Makrophagen und T-Lymphozyten als Glykoprotein gebildet und zu den Cytokinen gezählt. G-CSF wird als rekombinanter humaner Faktor Filgrastim (Neupogen®/Firma Amgen GmbH, CAS-Nr. 121181-53-1) bereits zur Behandlung von Neutropenien und neutropenischem Fieber eingesetzt. Weitere rekombinante humane G-CSF sind Lenograstim und Molgramostim. Eine neuroprotektive Wirkung von G-CSF wurde bisher noch nicht beschrieben.

[0006] An 24 Wistarratten wurde durch das international anerkannte Fadenmodell nach Longa et al eine 90 minütige Ischämie induziert. 30 Minuten nach Ischämieinduktion wurden 12 Ratten (n = 12) 2 ml NaCl intravenös über insgesamt 90 min infundiert; diese dienten als Kontrollgruppe (K). Die Therapiegruppe (T; n = 12) erhielt über denselben Zeitraum 20 Mikrogramm G-CSF in 2 ml NaCl gelöst. Vor Ischämieinduktion und 1, 2, 3, 4 und 24 Stunden danach wurde mittels ELISA (Biosource Europe, Fleurus, Belgien) die Konzentration von Interleukin 1-beta, IL-2, IL-6 und IL-10 bestimmt. Nach 24 Stunden wurden die Gehirne entnommen und von frontal 5 Hirnschnitte mit 2 mm Dicke angefertigt. Mittels TTC-Färbung wurde anhand von Schnitt 1, 2, 4 und 5 die Infarkt- und Hirnödemgrösse bestimmt. Schnitt 3 wurde weiter histologisch aufgearbeitet. Um die zerebrale Invasion mit neutrophilen Granulozyten nachzuweisen, wurde eine Myeloperoxidasefärbung MPO (DAKO, Carpinteria, CA, USA) durchgeführt. Durch Zugabe eines Anti-G-CSF-Antikörpers sollten Hinweise auf eine bisher noch nicht beschriebene Existenz des G-CSF-Rezeptors gefunden werden.

[0007] Zerebelläre Granulazellen wurden von P7-Mäusen gewonnen und nach einem etablierten Zellkulturmodell aufgearbeitet und gezüchtet. Nach 7 Tagen wurden die Granulazellen mit G-CSF behandelt und nach 30 min Glutamat zugegeben. Um die Überlebensrate der Zellen zu testen, wurde 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid (MTT, Sigma, München) 2 Stunden nach Glutamatstimulation appliziert. Nach weiteren 4 Stunden wurden die Zellen mit 1% SDS lysiert. Die Proben wurde bei 590 nm optischer Dichte gemessen. Des weiteren wurde mittels einer PCR der G-CSF-Rezeptor nachgewiesen werden. Dazu wurde aus Mäusegehirnen RNA extrahiert (RNA-kit, AGS, Heidelberg). 10 Mikrogramm RNA wurden mit MMLV reverser Transkriptase transkribiert. Für die PCR

wurden Primer von Exon 5 und 7 des G-CSF-Rezeptors benutzt (Ashihara, 1997). Die statistische Analyse erfolgte für das Tierexperiment nach Anova mit Bonferroni-Korrektur für multiple Gruppen.

[0008] Kontroll- und Therapiegruppe unterschieden sich nicht in den gemessenen physiologischen Parametern (BGA, HKT, Blutdruck und Körpergewicht). Nach 24 Stunden wurde eine leichte Erhöhung der im Blut vorhandenen neutrophilen Granulozyten festgestellt, die nicht signifikant war. Die Infarktgröße in den TTC-Schnitten betrug für die G-CSF-behandelte Gruppe (T) 6,7% +/–6,7% (n = 12) des Gesamthirnvolumens und war damit signifikant ($p < 0,05$) geringer als die der Kontrollgruppe mit 22,7% +/–6,3% (n = 12). Das errechnete Hirnödem war mit 4,7% +– 6,6% in der G-CSF-Gruppe ebenfalls signifikant ($p < 0,05$) geringer als in der nicht-behandelten Gruppe mit 12,0% +/–6,1%. Für alle gemessenen Interleukine bis auf IL-2 konnten signifikant geringere Serumwerte in der G-CSF-Gruppe nachgewiesen werden ($p < 0,05$). In der histologischen Auswertung des Hirnschnittes 3 konnte bei der MPO-Färbung für beide Tiergruppen lediglich eine Zunahme der Invasion von neutrophilen Granulozyten festgestellt werden, die mit der Größe des Infarktes zunahm. Signifikante Unterschiede wurden nicht beobachtet. An den Hirnschnitten konnte die Bindung von anti-Rezeptor-G-CSF sowohl an Neuronen als auch an Axonen und Dendriten nachgewiesen werden. In der Zellkultur konnte eine signifikante Abnahme des Zelluntergangs beobachtet werden. Dieser Effekt nahm mit steigender G-CSF-Dosis zu. Die PCR wies durch PT-PCR den Mausrezeptor im Hirngewebe nach. Das PCR-Produkt hatte die erwartete Größe von 567 bp und wurde durch PCR-Sequenzierung verifiziert.

[0009] Die Ergebnisse zeigen, dass G-CSF neuroprotektive Eigenschaften besitzt. Diese konnten sowohl im Tierexperiment über eine Verminderung des Infarktareals und Hirnödems in der G-CSF-behandelten Gruppe als auch an der Zellkultur durch einen mittels G-CSF verminderten Glutamatschaden bewiesen werden. Wirkmechanismen bestehen in einer Aktivierung des intrazerebralen G-CSF-Rezeptors, und eine Verminderung bestimmter Interleukine, die in die Entzündungsvorgänge bei zerebraler Ischämie eingreifen. Da G-CSF endogen produziert wird, einen zerebralen Rezeptor besitzt und neuroprotektive Eigenschaften aufweist, reiht es sich in die Reihe der Neurotrophine wie BDNF, IGF und NGF ein. Nebenwirkungen konnte in unserem Experiment nicht festgestellt werden.

[0010] Da G-CSF ein seit mehreren Jahren etabliertes Medikament mit geringer Nebenwirkungsrate ist, enthält unsere Anwendungsmöglichkeit bei zerebraler Ischämie eine große praktische Relevanz. G-CSF stellt einen wirkungsvollen pharmazeutischen Wirkstoff für die bisher unbefriedigende Schlaganfalltherapie dar.

Patentansprüche

1. Verwendung von G-CSF (Granulozyten Colony Stimulating Factor) zur Herstellung pharmazeutischer Präparate mit neuroprotektiver Wirkung zur Behandlung akuter Ischämien und neurodegenerativer Erkrankungen.
2. Verwendung nach Anspruch 1, dadurch gekennzeichnet, dass G-CSF ein Polypeptid und/oder ein Glycoprotein ist, und/oder ein Derivat und/oder ein Analogo von G-CSF ist, welches Granulocyten Zellkolonie stimulierende Aktivität hat.
3. Verwendung nach Anspruch 1 und 2, dadurch gekennzeichnet, dass G-CSF entweder chemisch-synthetisch (z. B. Derivate, Analoga, Isomere) und/oder rekombinant hergestellt und/oder aus G-CSF bildenden Zellen isoliert wird.
4. Verwendung nach Anspruch 1, dadurch gekennzeichnet, dass akute Ischämien wie z. B. bei Apoplexie, Schädel-Hirn-Trauma oder Tumoren behandelt werden.
5. Verwendung nach Anspruch 1, dadurch gekennzeichnet, dass neurodegenerative Erkrankungen wie z. B. Parkinson, Alzheimer behandelt werden.
6. Verwendung nach Anspruch 1, dadurch gekennzeichnet, dass das pharmazeutische Präparat fest, flüssig oder aerosolartig (z. B. Spray) ist.
7. Handelspackung enthaltend ein G-CSF haltiges pharmazeutisches Präparat zusammen mit Instruktionen für die Verwendung von G-CSF bei der neuroprotektiven Behandlung von akuter Ischämie oder neurodegenerativen Erkrankungen.

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GERMAN PATENT AND TRADEMARK OFFICE

12 **Offenlegungsschrift**
[Unexamined Application]

10 **DE 100 33 219 A1**

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21 Serial No.: 100 33 219.6
22 Application date: 7 July 2000
43 Date laid open: 24 January 2002

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Inventor will be named later

56 Cited references:
WO 9917798 A1
WO 0004926 A2
JP 05-24[?]885 A

The following text is taken from the documents filed by the Applicant

Request for examination per § 44 Patent Act has been filed

54 Neuroprotective effect of granulocyte colony stimulating factor (G-CSF)

57 The use of G-CSF (granulocyte colony stimulating factor) for synthesis of pharmaceutical preparations with neuroprotective effect for treatment of acute ischemias such as apoplexy and neurodegenerative diseases such as Parkinson's or Alzheimer's disease.

Description

[0001] The use of G-CSF (granulocyte colony stimulating factor) for synthesis of pharmaceutical preparations with neuroprotective effect for treatment of acute ischemias.

[0002] Strokes (apoplexia cerebri) are the third-most common cause of death in the western industrial countries. They are also one of the leading causes of permanent disability and need for nursing care and so – from the economic viewpoint – they constitute one of the most costly groups of diseases of all. In Germany, approximately 150,000 persons per year can now bc expected to suffer a stroke. Of those, 15 to 20 per cent die within the first four weeks. Only about one third of the surviving patients recover without serious lasting hindrance, whereas another third becomes permanently severely hindered by paralyses or other neurological conditions. In 80 per cent of the patients, the stroke results from a blood circulation disorder with subsequent ischemia in a local vascular territory. Blood circulation disorders in the brain are usually caused either macroangiopathically by thromboembolisms or hemodynamic retardation of blood flow, or microangiopathically in the small, intracerebral endarteries due to arteriosclerosis caused by blood pressure. In this context, the occurrence of strokes is favored by a series of risk factors, especially high arterial pressure, numerous heart diseases, which are associated with increased risk of embolisms – especially atrial fibrillation – diabetes mellitus, cigarette smoking, blood coagulation disorders and, to a lesser extent, high blood cholesterol. Embolic or thrombotic occlusion of one of the large arteries that supply the brain lead to territorial infarctions, which affect a local zone within the blood supply area of a given cerebral artery. The most frequent case is that of the supply area of the middle cerebral artery, involving a medial territorial infarction with corresponding "medial syndrome". This is also the most frequent of all manifestations of a stroke. Heretofore thrombolytic therapy has been promising only for selected patients. In recent years the diagnosis and therapy of acute cerebral ischemias has changed considerably due to new pathophysiological knowledge and methods. Thrombolysis offers the possibility of therapeutic intervention within a "therapeutic window" of 3 to 6 hours after an infarction has occurred. The objective is rapid resolution of the vascular occlusion and thus restoration of the cerebral blood circulation and improvement of the neurological symptoms. This is based on the pathophysiological idea that reopening of an occluded cerebral vessel

supports the preservation of hypoperfused, reversibly damaged brain tissue (otherwise known as ischemic penumbra) and thus restoration of neuronal functions. Heretofore, however, this treatment has been possible only in accredited neurological centers. Even the approval of rt-PA for strokes is still pending in Germany. Lysotherapy after 6 hours appears to lead to a particularly large number of adverse effects (increased number of intracranial hemorrhages) and should therefore be avoided. Other therapeutic procedures have not yet been evaluated. For the time being, various other substances are being investigated. They include in particular growth factors (bFGF) and drugs that block thrombocyte adhesion (anti-GP IIb/IIa, abcizimab). In recent years, numerous neuroprotective substances have been investigated in clinical studies. Unfortunately, although all of these substances tested so far have exhibited neuroprotective effects in animal experiments, none has shown any benefits in clinical practice. In particular, the glutamate antagonists, free radical scavengers and NMDA antagonists have not exhibited any clinical benefits, or to the contrary have even caused considerable adverse effects, which make clinical use impossible (psychoses, etc.). Other substances that prevent adhesion of leukocytes to vessel walls (anti-ICAM-1), or the inhibitor of glutamate-mediated NO synthetase (Lubeluzol) have not exhibited positive effects.

[0003] The object of the invention is to alleviate or eliminate the consequences of acute ischemia by administration of substances with neuroprotective effect. This object is achieved by the method having the features of claim 1.

[0004] The use of G-CSF (granulocyte colony stimulating factor) for synthesis of pharmaceutical preparations with neuroprotective effect for treatment of acute ischemias represents a successful therapeutic approach based on neuroprotective growth factors.

[0005] G-CSF, the English abbreviation for "granulocyte colony stimulating factor (G-CSF)", is an endogenous, hematopoietic growth factor that regulates the maturation, proliferation and differentiation of neutrophilic granulocytes. G-CSF is formed naturally as a glycoprotein by various monocytes, macrophages and T-lymphocytes, and is a member of the cytokines. G-CSF is already being used as the recombinant human factor known as filgrastim (Neupogen®/Amgen GmbH, CAS No. 121181-53-1) for the treatment of neutropenias and neutropenic fever. Further recombinant human G-CSFs are lenograstim and molgramostim. Heretofore a neuroprotective effect of G-CSF has not been described.

[0006] Using the internationally recognized thread model according to Longa et al., a 90-minute ischemia was induced in 24 Wistar rats. 30 minutes after ischemia induction, 12 rats (n = 12) received an intravenous infusion of 2 ml of NaCl within 90 minutes in total; these were classified as the control group (K). The therapy group (T, n = 12) received 20 micrograms of G-CSF dissolved in 2 ml of NaCl over the same period. Before ischemia induction and 1, 2, 3, 4 and 24 hours thereafter, the concentrations of interleukin 1-beta, IL-2, IL-6 and IL-10 were assayed by ELISA (Biosource Europe, Fleurus, Belgium). After 24 hours the brains were excised and 5 frontal brain sections of 2 mm thickness were prepared. The size of the infarction and cerebral edema was determined by TTC staining of sections 1, 2, 4 and 5. Section 3 was subjected to further histological conditioning. In order to demonstrate the cerebral invasion with neutrophilic granulocytes, myeloperoxide (MPO) staining was performed (DAKO, Carpinteria, CA, USA). By addition of an anti-G-CSF antibody, it was attempted to find indications of the existence of the G-CSF receptor, which has not been described heretofore.

[0007] Cerebellar granulocytes were obtained from P7 mice and conditioned and cultured according to an established cell-culture model. After 7 days the granulocytes were treated with G-CSF, then glutamate was added after 30 minutes. In order to test the survival rate of the cells, 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma, Munich) was applied 2 hours after glutamate stimulation. After a further 4 hours, the cells were lysed with 1% SDS. The optical density of the samples was measured at 590 nm. In addition, the G-CSF receptor was detected by means of PCR. For this purpose, RNA was extracted from mouse brains (RNA kit, AGS, Heidelberg). 10 micrograms of RNA was transcribed with MMLV reverse transcriptase. For the PCR, primers of exon 5 and 7 of the G-CSF receptor were used (Ashihara, 1997). The statistical analysis for the animal experiment was performed according to Anova with the Bonferroni correction for multiple groups.

[0008] The control and therapy groups did not differ in the measured physiological parameters (BGA, HKT, blood pressure and body weight). After 24 hours, a slight but non-significant elevation of the neutrophilic granulocytes present in the blood was observed. The infarction size in the TTC sections was 6.7% ± 6.7% (n = 12) of the total brain volume for the group (T) treated with G-CSF, and thus was significantly ($p < 0.05$) smaller than that of the control group with 22.7% ± 6.3% (n = 12). The calculated cerebral edema was also significantly ($p < 0.05$) smaller

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(4.7% ± 6.6%) in the G-CSF group than in the untreated group (12.0% ± 6.1%). For all measured interleukins except IL-2, significantly lower serum levels were found in the G-CSF group ($p < 0.05$). In the histological evaluation of brain section 3 after MPO staining, only an increase – which became larger with infarction size – of the invasion of neutrophilic granulocytes was found for both animal groups. Significant differences were not observed. In the brain sections, binding of anti-receptor G-CSF not only to neurons but also to axons and dendrites was detected. In the cell culture, a significant decrease of cell death was observed. This effect became more pronounced with increasing G-CSF dose. By means of PT-PCR, the PCR revealed the mouse receptor in the brain tissue. The PCR product had the expected size of 567 bp and was verified by PCR sequencing.

[0009] The results show that G-CSF has neuroprotective properties. These were demonstrated both in the animal experiment, through a reduction of infarction area and of cerebral edema in the group treated with G-CSF, and in the cell culture, where glutamate damage was reduced by means of G-CSF. The mechanisms of action comprise activation of the intracerebral G-CSF receptor and reduction of certain interleukins, which contribute to inflammation processes in cerebral ischemia. Since G-CSF is produced endogenously, has a cerebral receptor and exhibits neuroprotective properties, it is a member of the neurotrophin series, such as BDNF, IGF and NGF. Adverse effects were not found in our experiments.

[0010] Since G-CSF is a drug that was established several years ago and that has few adverse effects, our ability to use it for cerebral ischemia has great practical relevance. G-CSF represents an effective pharmaceutical ingredient for stroke therapy, which heretofore has been unsatisfactory.

Claims

1. The use of G-CSF (granulocyte colony stimulating factor) for synthesis of pharmaceutical preparations with neuroprotective effect for treatment of acute ischemias and neurodegenerative diseases.

2. The use according to claim 1, characterized in that G-CSF is a polypeptide and/or a glycoprotein, and/or a derivative and/or an analog of G-CSF, which has granulocyte colony stimulating activity.

3. The use according to claim 1 and 2, characterized in that G-CSF is synthesized either by chemical techniques (in the form of derivatives, analogs, isomers) and/or by recombinant techniques, and/or is isolated from cells that form G-CSF.

4. The use according to claim 1, characterized in that there are treated acute ischemias, such as apoplexy, cranial brain trauma or tumors.

5. The use according to claim 1, characterized in that there are treated neurodegenerative diseases such as Parkinson's and Alzheimer's.

6. The use according to claim 1, characterized in that the pharmaceutical preparation is a solid, liquid or aerosol (such as a spray).

7. A commercial pack comprising a pharmaceutical preparation containing G-CSF together with instructions for use of G-CSF in neuroprotective treatment of acute ischemia or of neurodegenerative diseases.

JUL 3 0 2007

DOCKET NO: 242650US0CONT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF	:
WOLF-RUEDIGER SCHAEBITZ, ET AL.	: EXAMINER: BORGEEST
SERIAL NO: 10/659,295	:
FILED: SEPTEMBER 11, 2003	: GROUP ART UNIT: 1649
FOR: METHODS OF TREATING NEUROLOGICAL CONDITIONS WITH HEMATOPOIETIC GROWTH FACTORS	:

DECLARATION UNDER 37 C.F.R §1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

Mr. Wolf-Ruediger Schäbitz states that:

1. Wolf-Ruediger Schäbitz, Stefan Schwab, and Rainer Kollmar are named coinventors of the above-identified application.

2. That Wolf-Ruediger Schäbitz has been employed by the University of Heidelberg for 8 years as a physician in the field of Neurologie.

3. The relevant disclosure of using GCSF for the treatment of stroke and other indications in DE 100 33 219 A1 published January 24, 2002 as it relates the claims of the above-referenced application pending in the United States Patent Office is the own work of Wolf-Ruediger Schäbitz, Stefan Schwab, and Rainer Kollmar.

4. The undersigned declare further that all statements made herein are of his own knowledge are true and that all statements made on information are believed to be true. Further that these statements were made with the knowledge that willful false statements and

the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Signature

Date 14.6.07

OIPE IAP59 JUL 30 2007 PATENT & TRADEMARK OFFICE

DOCKET NO: 242650US0CONT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF	:
WOLF-RUEDIGER SCHAEBITZ, ET AL.	: EXAMINER: BORGEEST
SERIAL NO: 10/659,295	:
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DECLARATION UNDER 37 C.F.R §1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

Mr. Stefan Schwab states that:

1. Wolf-Ruediger Schäbitz, Stefan Schwab, and Rainer Kollmar are named coinventors of the above-identified application.

2. That Stefan Schwab has been employed by _the University of Heidelberg_ for _15___ years as a ____staff member/M.D.___ in the field of __clinical Neurology__

3. The relevant disclosure of using GCSF for the treatment of stroke and other indications in DE 100 33 219 A1 published January 24, 2002 as it relates the claims of the above-referenced application pending in the United States Patent Office is the own work of Wolf-Ruediger Schäbitz, Stefan Schwab, and Rainer Kollmar.

4. The undersigned declare further that all statements made herein are of his own knowledge are true and that all statements made on information are believed to be true. Further that these statements were made with the knowledge that willful false statements and

the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Signature [signature]

Date 14. 6. 07

DOCKET NO: 242650US0CONT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF	:
WOLF-RUEDIGER SCHAEBITZ, ET AL.	: EXAMINER: BORGEEST
SERIAL NO: 10/659,295	:
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DECLARATION UNDER 37 C.F.R §1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

Mr. Rainer Kollmar states that:

1. Wolf-Ruediger Schäbitz, Stefan Schwab, and Rainer Kollmar are named coinventors of the above-identified application.

2. That Rainer Kollmar has been employed by the university of Heidelberg for 7 years as a neurology fellow in the field of Neurology.

3. The relevant disclosure of using GCSF for the treatment of stroke and other indications in DE 100 33 219 A1 published January 24, 2002 as it relates the claims of the above-referenced application pending in the United States Patent Office is the own work of Wolf-Ruediger Schäbitz, Stefan Schwab, and Rainer Kollmar.

4. The undersigned declare further that all statements made herein are of his own knowledge are true and that all statements made on information are believed to be true. Further that these statements were made with the knowledge that willful false statements and

the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Signature

Date 15-July Juni-07

DOCKET NO: 242650US0CONT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF	:
WOLF-RUEDIGER SCHAEBITZ, ET AL.	: EXAMINER: BORGEEST
SERIAL NO: 10/659,295	:
FILED: SEPTEMBER 11, 2003	: GROUP ART UNIT: 1649
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DECLARATION UNDER 37 C.F.R §1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

Armin Schneider states that:

1. I am a named coinventor of the above-identified application.
2. That I have been employed by Sygnis (former Axaron) for 8 years as a physician in the field of Neuroscience.
3. The following experiments were performed by me or under my supervision.
4. The following experiments demonstrate that G-CSF acts to improve outcome after experimental spinal cord injury (SCI) by inhibiting neuronal cell death.
5. We used the following in vivo model for experimental SCI. Female C57BL/6 wild-type mice at 2 months of age were anesthetized. After laminectomy at the vertebral level Th8/9, the spinal cord was dorsally transected to approximately 80% with fine iridectomy scissors leaving a ventral tissue bridge intact.

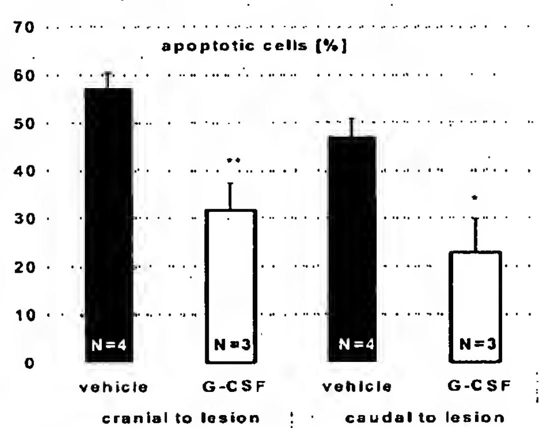
In the treatment group, the mice received i.v. G-CSF (60 µg/kg bodyweight Neupogen; Amgen) 5 min after the experimental SCI, followed by continuous s.c. application over 2 weeks (30 µg/kg bodyweight) via an osmotic minipump. The experimental outcome after SCI of the G-CSF treated mice were compared with the one of sham treated mice. All animal experiments were performed in a fully blinded and randomized fashion.

Neuronal apoptosis following SCI was evaluated using a TUNEL assay. 3 days after SCI, spinal cords of mice were extracted and embedded in paraffin. Sections of the spinal cord were stained with the Apo-BrdU IHC Kit (Chemicon). TUNEL signal was visualized with streptavidin-coupled Alexa Fluor 555 (1:200). Nuclei were counterstained with Hoechst33342 (1:10,000). BrdU-positive (apoptotic) as well as total nuclei were counted in representative fields of the sections. Means of the ratios of apoptotic to total nuclei were calculated for each animal (n=3-4 per group, G-CSF and sham treated).

The functional outcome in the experimental SCI depending on the treatment with G-CSF or sham was analyzed with behavioral tests such as BBB locomotor score (in brief, the mice were placed on a runway and hindlimb locomotion was scored from 0 (for no observable hindlimb movement) to 21 points (regular movement)), grid walk test (in brief, the ability to navigate across a 1m long runway with irregularly spaced metal bars and gaps was scored by counting the number of errors in foot placement), and swimming score(in brief, the locomotor performance was determined in absence of cutaneous and proprioceptive sensory input from the hindlimbs. by scoring hindlimb movement, hindlimb-forelimb coordination, tail position, paw position, and lateral stability).

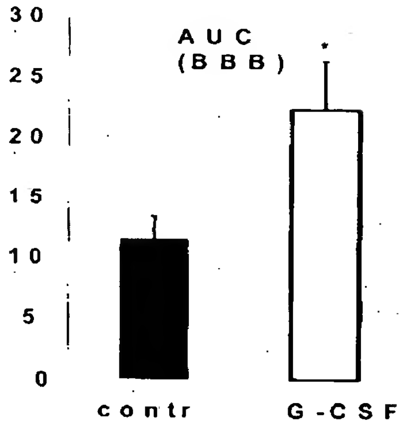
6. We observed the following results.

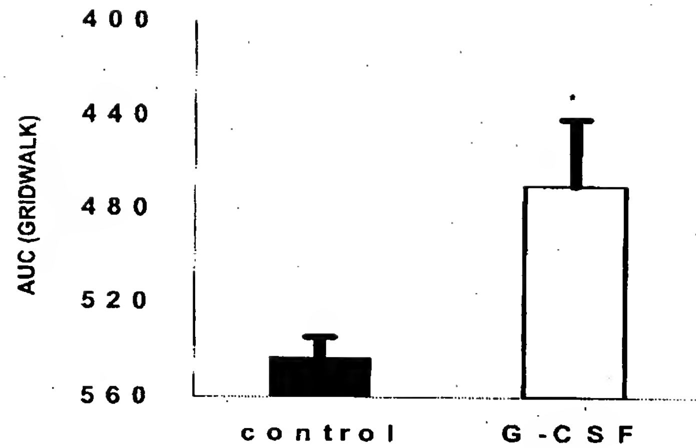
As apoptosis appeared maximal at 3 days after SCI, we determined the extent of programmed cell death at the cellular level using a sensitive TUNEL staining method. The percentage of apoptotic nuclei was determined in the vicinity of the lesion (cranial and caudal to the lesion border) in representative fields in the ventral, central, and dorsal third of the spinal cord. There was a high percentage of TUNEL-positive nuclei both cranial and caudal to the lesion (57% or 47%, respectively). This number was almost halved by G-CSF treatment (31% and 23%, respectively)

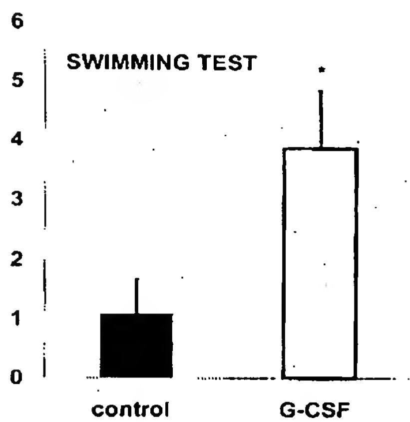


The functional outcome of the G-CSF and the sham treated mice following the SCI was analyzed using the behavioral tests BBB locomotor score and grid walk test during the first 5 weeks after SCI. These time series data were subjected to an "area under the curve" (AUC) analysis. Further, the swimming score was determined once at 5 weeks following SCI.

All these behavioral tests demonstrated a highly significant benefit in the G-CSF-treated group.







7. Using an experimental SCI in vivo model we were able to demonstrate that G-CSF acts to decrease apoptosis triggered by the SCI. Apoptosis of both neurons and oligodendrocytes is one important primary pathophysiological mechanism in SCI. Accordingly, we also observed a clear improvement in functional motor outcome due to G-CSF treatment after SCI by a number of criteria. SCI and TBI both refer to injuries of the central nervous system (CNS) and accordingly can be subsumed as neurotrauma of the CNS. Whereas TBI refers to the brain tissue itself SCI refers to the spinal cord, both components of the central nervous system. TBI and SCI have many physiological and pathophysiological mechanisms (e.g. apoptosis of CNS neurons and oligodendrocytes) in common. Therefore, I conclude that the SCI model presented here is also predictive for TBI.

8. These data demonstrate that G-CSF treatment after experimental SCI as an in vivo model for neurotrauma of the CNS reduces the apoptotic processes subsequent to the injury and thereby improves the functional outcome. These observations are consistent with the data presented in the specification (e.g. the neuroprotective and anti-apoptotic effect of G-CSF) and show that G-CSF is an effective treatment for neurotrauma of the CNS, i.e. spinal cord injuries and traumatic brain injuries.

9. The undersigned declare further that all statements made herein are of his own knowledge and are true and that all statements made on information are believed to be true: Further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Signature

24.7.2007

Date